

SORTASE A CONJUGATION

Aim

To conjugate two proteins using the enzyme Sortase A.

Material

1. 10 ml of 521 μM Z_{Q32C} -SR- H_6 or combat protein dissolved in 1x Sortase A binding buffer containing 50 mM Tris base, 150 mM NaCl, 10 mM CaCl_2 , pH 7.5.
2. 25 ml 10x Sortase A binding buffer (0.5 M Tris base, 1.5 M NaCl, 100mM CaCl_2 , pH 7.5)
3. 2 tubes with 50 μl of 1mM Sortase A in Sortase A storage buffer

Procedure

In a 1.5 ml tube add:

1. 30 nmol of the protein with the N terminal Gly residue (e.g. spider silk)
2. 120 nmol (4x excess) of the protein with C-terminal LPETGG- H_6 -motif (e.g. a combat protein or Z_{Q32C} -SR- H_6)
3. Add 10x Sortase A binding buffer so the final concentration is 1x (50 mM Tris base, 150 mM NaCl, 10mM CaCl_2 , pH 7.5)
4. Add Sortase A to the final concentration of 5 μM
5. Let the reaction proceed for 30 minutes at 37°C or 3 hours at room temperature.
6. Analyse by SDS-PAGE

Note!

Keep the total volume as low as possible, 500 μl if possible, but it depends on the concentration of the proteins being used.

Sources

This protocol was given to iGEM Stockholm by one of the advisors, Kristina Westerlund at Albanova. It was modified to suit the proteins and equipment.